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14. ABSTRACT
There are ~300,000 individuals in the United States with spinal cord injury (SCI), where ~22% are veterans [1,2]. While their quality of life is significantly affected by lower urinary tract symptoms (LUTS), most treatments are palliative or ineffective. We focused on the therapeutic benefits of β3 adrenoceptor agonists, botulinum neurotoxin type A (BTX-A) intradetrusor injections and their combination. BTX-A inhibits neurotransmitter release from nerve terminals. This has therapeutic effects on bladder dysfunction by inhibiting parasympathetic nerves to decrease reflex contractions, and afferent nerves, to reduce sensory symptoms. However, by also inhibiting sympathetic nerves, BTX-A decreases norepinephrine release and stimulation of detrusor β_3 adrenoceptors thereby decreasing relaxation and bladder compliance. Accordingly, we assessed the effects of BTX-A and β_3 adrenoceptor agonists in combination. In control mice, β3 agonists had little effect as β3 adrenoceptors are not normally expressed in mice. In SCI mice, β3 agonists were beneficial by abolishing intrinsic bladder contractions and enhancing bladder compliance suggesting that β3 adrenoceptors are upregulated in pathology. In BTX-A treated SCI mice, β3 agonists significantly improved bladder compliance compromised by the toxin. Thus, β3 adrenoceptor agonists in combination with BTX-A are beneficial in improving bladder function in SCI patients.

15. SUBJECT TERMS
Lower urinary tract symptoms (LUTS), spinal cord injury (SCI), Botulinum Toxin Type A and β3 adrenoceptor agonists

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TABLE OF CONTENTS

INTRODUCTIONS

There are an estimated 250,000 to 400,000 individuals in the United States with spinal cord injury/disease, where 22% are military veterans [1, 2]. From this subset of individuals, approximately 40% would have received the injury during active service. While the quality of life for these individuals is considerably affected by lower urinary tract dysfunction, most treatments are only palliative and so there is a significant need for further research to develop improved treatments for these patients. Accordingly, our studies are designed to further our understanding of lower urinary tract complications associated with acute and chronic spinal cord injury, with the overriding goal being to improve the quality of life for these patients through improved treatment methods.

Spinal cord injury can have significant consequences on lower urinary tract function. In upper lesions (above thoracic level, T12), the sacral micturition center and sacral reflex arc remain intact and continue to function, but are disconnected from supraspinal control centers. This can result in detrusor-sphincter-dyssynergia (DSD) where patients are unable to void efficiently due to discoordination of the contraction of the bladder and relaxation of the urethral sphincters. This can cause urinary retention, hypertrophy and damage to the bladder and kidneys. However, there is partial recovery of bladder voiding function after spinal cord injury (SCI) which has been attributed to remodeling of the neural connections within the spinal cord [3].

β_3-aderenoceptors have shown promise as therapeutic targets for treating bladder overactivity. They are highly expressed throughout the human bladder and their activation relaxes detrusor smooth muscle [4, 5]. While these receptors are thought to be the main target of β3 agonist therapy for bladder overactivity [6, 7, 8], the effects of their stimulation on bladder sensory function have yet to be fully elucidated.

BTX-A has therapeutic effects on bladder dysfunction by inhibiting acetylcholine release from parasympathetic nerves. This decreases reflex contractions and inhibits neuropeptide release from afferent nerves which reduces sensory symptoms. However, BTX-A inhibition of sympathetic nerves may decrease norepinephrine release and stimulation of detrusor β3 adrenoceptors in humans to adversely affect bladder compliance.

BODY

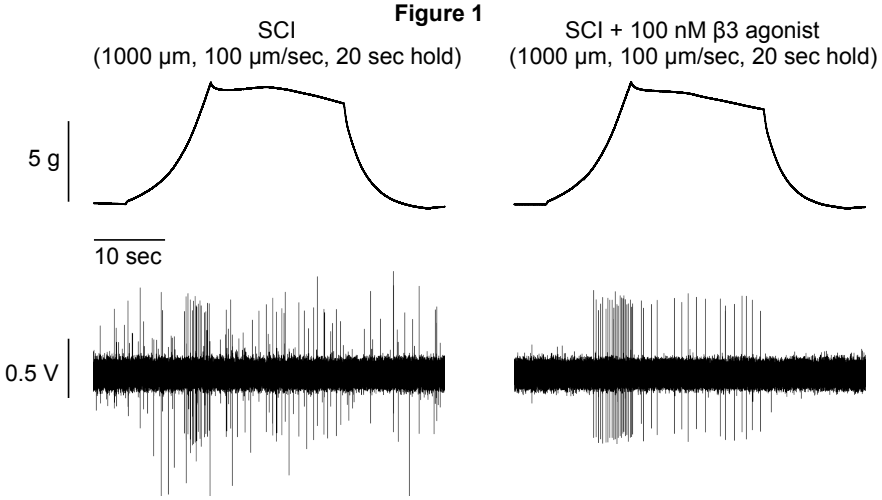
In these studies, we evaluated the effect of a β3 adrenoceptor agonist (BRL-37344) on urinary bladder function and afferent nerve activity in control and spinal cord injured (SCI, T8-T9) mice. We have also assessed the effect of BTX-A and β3 adrenoceptor agonist combination therapy.

Adult female C57Bl/10 mice were used for *in vivo* decerebrate cystometry and *in vitro* bladder-pelvic spinal nerve (L6-S1) recordings. For nerve recordings, bladders were connected to a tension transducer and spinal nerves were passed into adjacent oil recording chambers. The bladders were stretched *via* a computer-controlled stepper motor to evoke mechanosensitive firing. The β3 agonist (10nM – 1μM) was added to the perfusate. For cystometry, the β3 agonist or the β3 antagonist (L-748,337) were given IP at 0.5 mg/kg. All the studies were done in the presence of 100nM propranolol to block β1 and β2 adrenoceptors.

BTX-A was injected (2 units) *in vivo* into mouse bladder walls. After 48 hours, the animals were used for cystometry or their bladders were excised for nerve recordings. When injected IP, 1 unit of BTX-A is, by definition, the LD_{50} in mice. However, when injected into the bladder wall, 2 units are not lethal but decrease nerve mediated contractions by 70%.

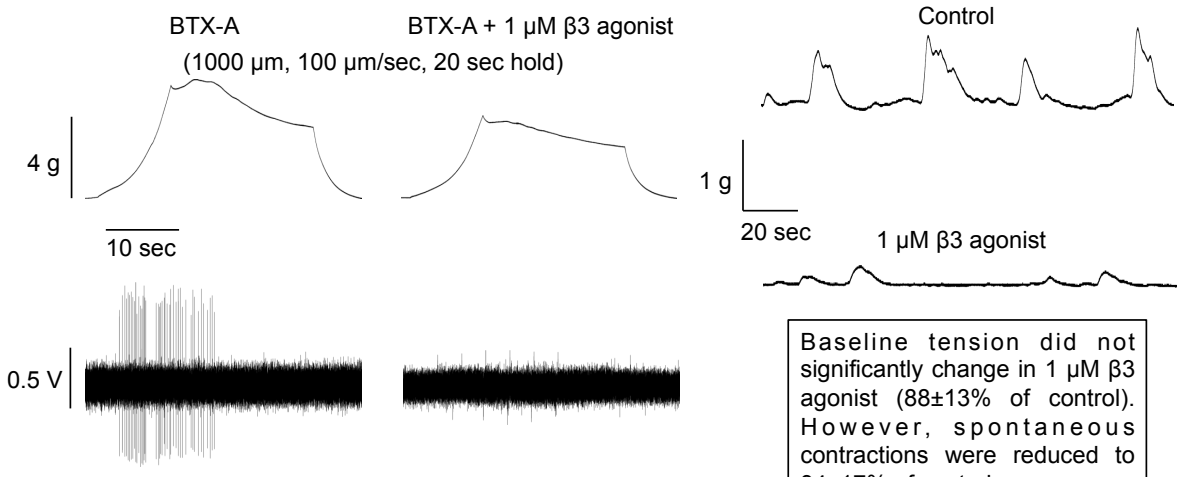
In control mice, addition of β3 agonist did not alter stretch-evoked afferent activity (not shown). However, SCI mice exhibit large amplitude spontaneous detrusor contractions and afferent firing not seen in controls. The spontaneous activity was eliminated by β3 agonist while stretch-evoked firing was not affected (**Figure 1**).

Figure 1



The efficacy of β3 agonist was enhanced in SCI rodents treated with BTX-A (**Figure 2**).

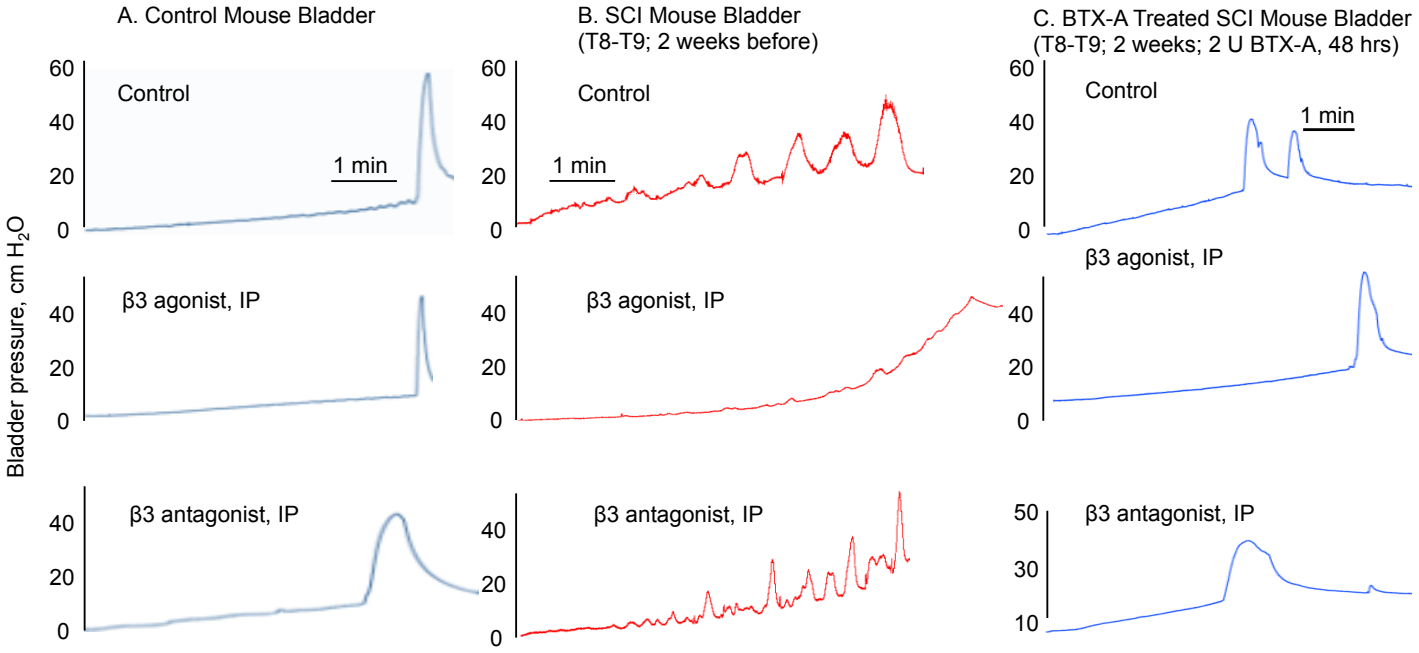
Figure 2



Baseline tension did not significantly change in 1 μM β3 agonist (88±13% of control). However, spontaneous contractions were reduced to 34±17% of control.

In cystometric studies of the bladders of untreated control mice, the β3 agonist was without effect (**Figure 3A**). However, in SCI mice, β3 adrenoceptor agonist was beneficial by abolishing intrinsic bladder contractions and increasing compliance (**Figure 3B**). In BTX-A treated SCI mice, β3 agonist dramatically improved bladder compliance which was decreased by the toxin (**Figure 3C**).

Figure 3



The results in figure 3 suggest that β3 adrenoceptors do not have a significant role in normal mouse bladders and that bladder relaxation in these animals may be mediated by the β2 or β1 receptor subtype. Following SCI, however, our data suggest that β3 receptors are upregulated. While the β3 subtype predominates over β1 and β2 in human bladders, it may also be upregulated in pathology thereby increasing the efficacy of β3 agonists. Moreover, in SCI patients treated with BTX-A, combination with a β3 adrenoceptor agonist may be beneficial by improving bladder function.

KEY RESEARCH ACCOMPLISHMENT

- β3 adrenoceptor agonists inhibit nociceptive but not stretch-sensitive afferent nerves. This should decrease painful nociception without adversely affecting stretch-evoked micturition.

- BTX-A inhibits nociceptive and stretch-sensitive afferent nerves. While this will decrease painful sensation, given that BTX-A also inhibits parasympathetic nerves, this may inhibit bladder contraction requiring catheterization.

- β3 adrenoceptor agonists inhibit spontaneous bladder contractions which may also decrease nociception.

- β3 adrenoceptors are normally absent in mouse bladders but upregulated following SCI. While these receptors are present in human bladders, they may also be upregulated in pathology making β3 adrenoceptor agonists more efficacious.

- β3 adrenoceptor agonists increase bladder compliance while BTX-A decreases it. Accordingly, in cases where BTX-A is used, combination with a β3 adrenoceptor agonist may be therapeutically beneficial.

REPORTABLE OUTCOMES

Presentations at the International Consultation on Incontinence – Research Society (ICI-RS) meeting:

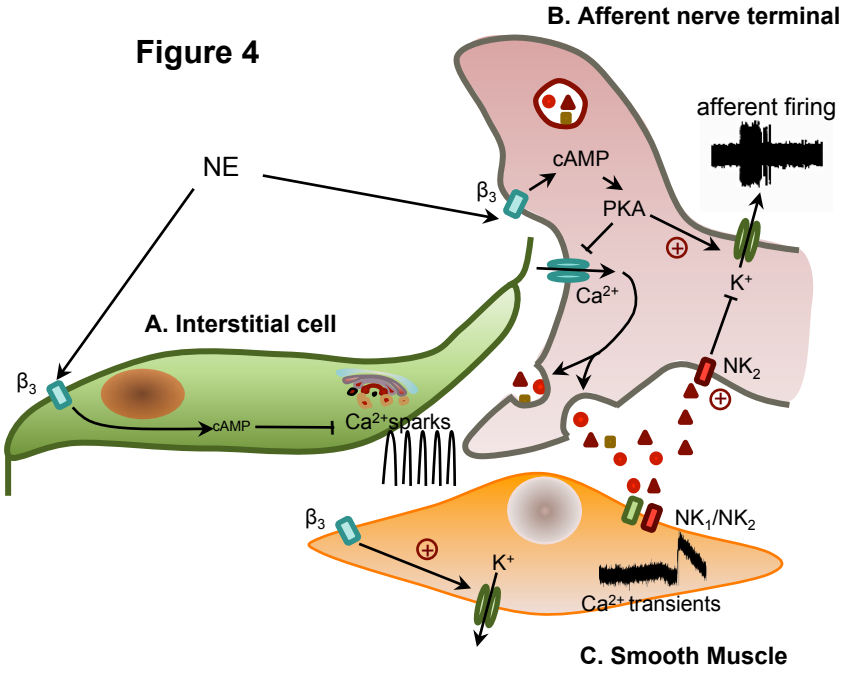
1. Kanai AJ, Ikeda Y, Hanna-Mitchel A. Do we understand any more about LUT interstitial cells?
2. Zabbarova IV, Gajewski J. Does our limited knowledge of the mechanisms of neuromodulation limit its benefits for patients

Manuscripts:

1. Y. Ikeda, I.V. Zabbarova, L.A. Birder, W.C. de Groat, C.J. McCarthy, A.T. Hanna-Mitchell, A.J. Kanai. Botulinum neurotoxin serotype A suppresses neurotransmitter release from afferent as well as efferent nerves in the urinary bladder. Eur. Urol. Mar 23, (2012).

CONCLUSION

Our results demonstrate that β3 adrenoceptor agonists increase bladder compliance while BTX-A decreases it. Accordingly, in cases where BTX-A is used, combination with a β3 agonist may be therapeutically beneficial. The putative sites and mechanisms of action of these receptors in the LUT are shown in figure 4 below.



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APPENDICIES

http://www.ncbi.nlm.nih.gov/pubmed/22480459